Effects of cell cycle inhibitor p27 on skeletal muscle stem cell and muscle regeneration

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ABSTRACT

Duchenne Muscular Dystrophy (DMD) is a progressive muscle disorder resulting in the loss of the dystrophin-associated complex and thus muscle damage due to the absence of dystrophin. Possible therapies include muscle stem cell transplantation into DMD patients, which may potentially replace the damaged muscle tissue. As such, muscle stem cells, or satellite cells, migrate, activate their cell cycle to rapidly divide, and fuse with existing fibers to serve as a form of muscle repair. The Asakura lab and other groups previously identified p27, a cell cycle inhibitor, in quiescent satellite cites, which is rapidly down-regulated during activation of the cell cycle in satellite cells. Therefore, there is potential that modification of expression of regulatory genes, such as p27, may play an essential role in maintaining quiescent satellite cells. This project focuses on studying p27 and its role in muscle regeneration, as well as future benefits to cell therapy for DMD patients.

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is a muscle disorder, in which absent or deficient dystrophin results in the loss of the dystrophin-associated complex that binds to the extracellular matrix, as well as other functional abnormalities of muscle surface membranes, thus leading to progressive muscle damage (Bonilla, et al, 1988). Several therapies to combat this disorder by introducing functional dystrophin include cell therapy, gene therapy, or a combination of the two. It is possible that stem cells transplanted into DMD patients will have the potential to replace damaged tissue. Satellite cells are, mitotically quiescent, muscle stem cells localized beneath the basal lamina of the muscle fiber. Upon muscle damage, they migrate out, activate their cell cycle, rapidly divide and fuse with the existing fibers. A population of these cells migrates back beneath the basal lamina to become quiescent satellite cells, serving as a form of self-renewal (Motohashi, et al, 2014). Satellite-cell-derived myoblasts can be

isolated from adult skeletal muscles and expanded ex vivo for transplantation into mice muscle and DMD patients. The Asakura lab had previously identified expression of p27, a cell cycle regulator at the G1 phase, in quiescent satellite cells and differentiating myocytes, and these genes are quickly down-regulated during satellite cell cycle activation. Likewise, it was found, through examination of differentiation progression in skeletal muscle, that cell cycle arrest was necessary in proliferating myoblasts, after which the myoblasts differentiate into post-mitotic myocytes to go on and fuse with each other to form multinucleated myotubes (Mohan et al., 2017). Thus, cell cycle inhibitors have a fundamental role in the differentiation and development of muscle fibers. It is possible that modification of gene expression for key regulatory genes, such as p27, in myoblasts would be essential in cell therapy for DMD as evidenced in MyoD mutant myoblasts (Asakura, et al., 2007). As such, I hypothesized that p27 may play critical roles in maintenance of quiescent satellite cells and muscle regeneration. The purpose of this project was to evaluate whether p27 is required for proper muscle regeneration and satellite cell function by assessing muscle diameters after Hematoxin-Eosin staining.

MATERIALS AND METHODS

Prior to the start of this project, p27 gene KO mice have already been established in the Asakura laboratory. Genotyping to detect the p27 gene KO mice was performed by PCR using specific primers. All protocols were approved by the Animal Care and Use Committee of the University of Minnesota. The p27 gene KO mice were viable and had survived until adulthood. 50 µl of 10 µM cardiotoxin was injected into one tibialis anterior (TA) muscle to induce muscle regeneration in p27 wild-type and p27 KO mice. Saline was injected into the other TA muscle for control experiments. The TA muscles were harvested one to two weeks after injection to compare muscle cryosections between the control and cardiotoxin-injected muscles after Hematoxin-Eosin (HE) staining. HE staining was used to assess general histology of the muscle cryosections. Images were captured using a digital camera attached to a microscope, and images were processed with Adobe Photoshop CS2. Image J was used to measure and analyze the diameter data of the muscle fibers in each cryosection.

RESULTS

To reveal whether p27 plays essential roles in muscle regeneration, we first compared muscle histology between p27 wild-type and KO mice. Our results clearly indicated that when analyzing the p27 KO and wild-type mice that had received no cardiotoxin, there was a similar distribution of small and large fibers in both genotypes of mice (Fig. 1). The T-Test revealed that there were no significant differences in muscle fiber size between p27 KO and wild-type mice.

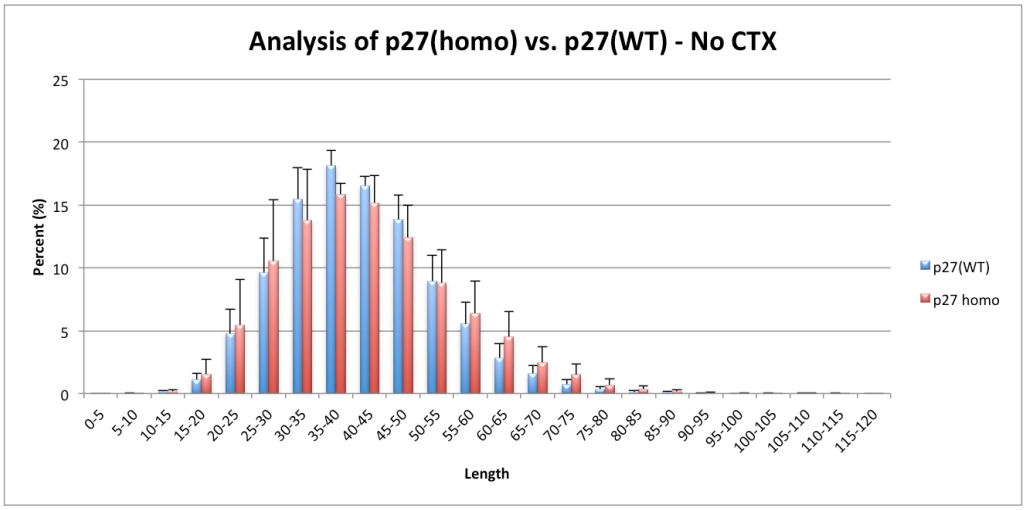

Figure 1: The distribution of muscle fiber lengths is similar for both the p27 wild-type and p27 KO mice. The average length of muscle fibers is similar for both types of mice without cardiotoxin injection as well.

By contrast, in the p27 KO mice, there were larger fibers by day 7 and day 12 after cardiotoxin injection compared to the wild-type mice. In other words, the average size of the muscle fiber in p27 KO mice was larger than the average size of muscle fibers in the wild-type mice at 7 and 12 days after cardiotoxin injection (Figs. 2 and 3). It was also noted that the difference in average size of muscle fibers between the p27 wild-type and KO mice was greater day 7 after injection compared to day 12 after injection.

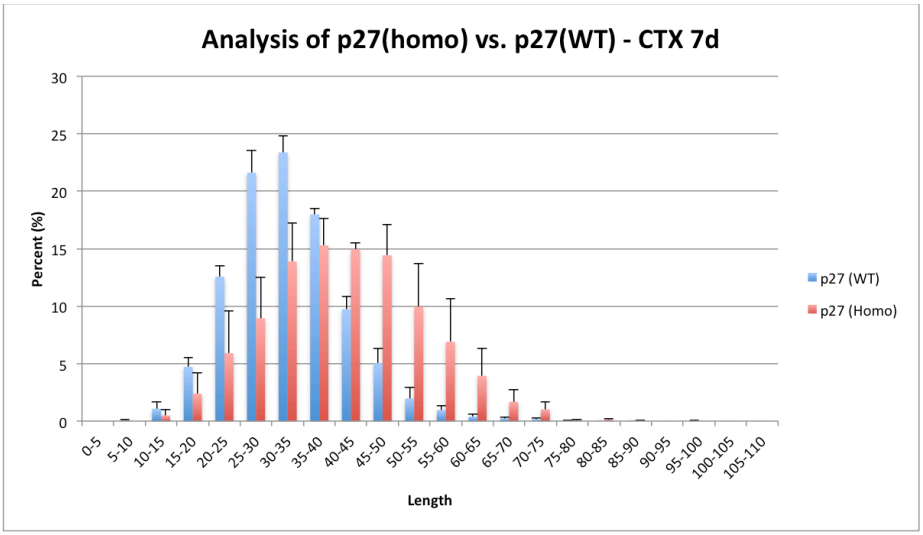

Figure 2: The distribution of muscle fiber size for the p27 KO mice is more greatly spread on the larger end of the scale, thus p27 KO mice regenerated muscle at a greater rate compared to the p27 wild-type mice.

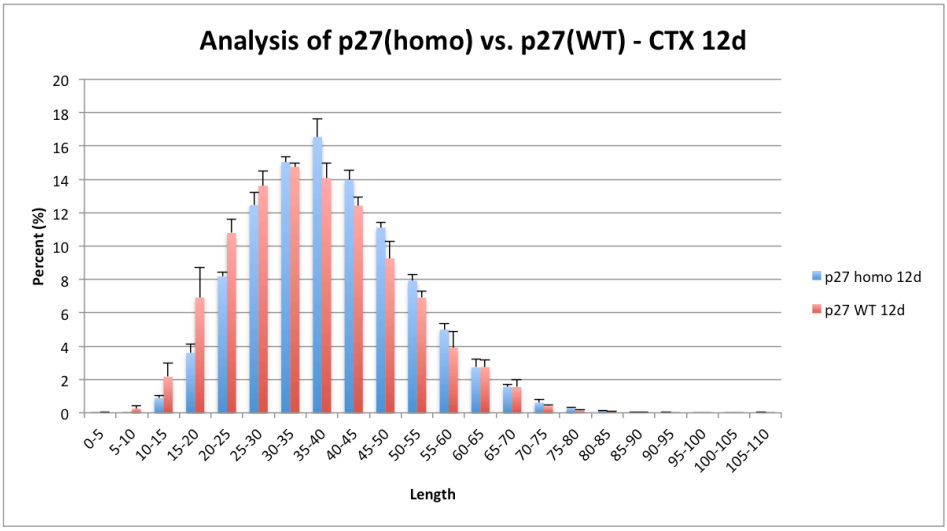

Figure 3: Though not as evident than on day 7 after injection, the p27 KO mice muscle fibers are still slightly larger than the wild-type mice muscle fibers.

However, by day 30 after injection, this tendency was not true, as there were a larger number of smaller fibers in the p27 KO mice compared to the wild-type mice.

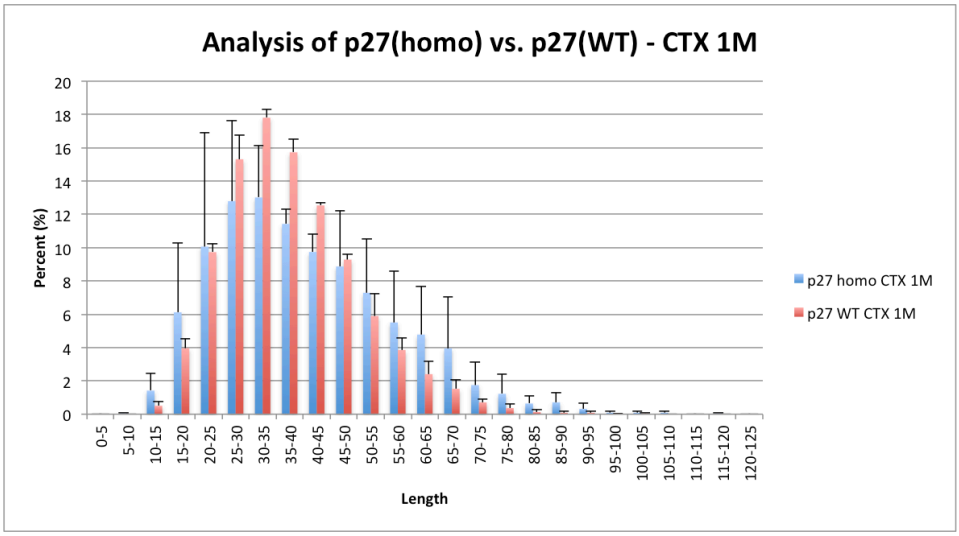

Figure 4: There appears to be a larger number of smaller fibers in the wild-type mice than the KO mice.

As indicated above, it was evident that the average size of the muscle fiber in the p27 wild-type sample was larger than the average size of the muscle fiber in the p27 KO mice one month after injection.

DISCUSSION

Analysis of our muscle regeneration assay after cardiotoxin-mediated muscle injury revealed that lacking p27 allowed for more rapid muscle regeneration by day 7 and 12 after cardiotoxin injection, thus indicating that the p27 gene KO myogenic precursor cells may expand more and increase the cell number before differentiation. In theory, these results revealed that in the short term the p27 KO mice were better at regeneration than the wild-type mice. However, in the long term after injury, the p27 wild-type muscle fibers began to maturate and become larger than the p27 KO mice. Therefore, it can be concluded that p27 plays a critical role in muscle fiber maturation during muscle regeneration, while lacking p27 resulted in immediate activation of satellite cell cycles to promote increased number of myogenic precursor cells after cardiotoxin injection, causing promotion of early phase of muscle regeneration. In other words, the absence of p27, a cell cycle inhibitor, resulted in the lack of satellite cell cycle arrest at the G1 phase, as evidenced by the increased number of myogenic precursor cells after injection in the KO mice. Previous research indicated that p27 KO mice had a hyper-proliferative

phenotype, characterized by enlarged organs, which was supported by the overall large size of the muscle fibers immediately after cardiotoxin injection in the p27 KO mice in this assay (Mohan et al., 2017). Therefore, there is potential that modification of p27 gene may facilitate muscle regeneration, and thus future benefits to cell and/or gene therapy for DMD patients.

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